

PROJECT NUMBER: 1730
PROJECT TITLE: Plant, Cell & Tissue Culture Research
PROJECT LEADER: I. L. Uydess
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PERIOD COVERED: October, 1988

I. TOBACCO-IDENTICAL PRESERVATIVES

A. Objective: To develop procedures and to establish microbiological screens for the evaluation of new, nature-identical preservatives as replacements for and/or as adjuncts to propylparaben.

B. Status:

Phase I Screens:

Potassium sorbate has been evaluated in the Phase I shake-flask assay against propylparaben, benzoic acid and decanoic acid for antimicrobial activity against *B. coagulans* (PM-13). Potassium sorbate at 1000 µg/ml produced a half-maximal inhibition of growth similar in effect to that of the 150 µg/ml paraben control and 500 µg/ml benzoic acid. The 50 µg/ml decanoic acid control completely inhibited the growth of the target organism, PM-13.

An assay to assess the efficacies of a decanoic acid-propylparaben mixture and of a decanol-propylparaben mixture (each component at a 25 µg/ml level) was performed. The decanol-propylparaben mixture completely inhibited bacterial growth of PM-13 while the decanoic acid-propylparaben mixture was not completely inhibitory. The greater solubility of decanol probably accounts for these results.

Phase II Screens:

A dose-response assay applying decanoic acid in Park 500 SEL was conducted in the Phase II screen. It now appears evident that 300 to 400 µg/ml of decanoic acid provides approximately the same antimicrobial activity with regard to inhibiting many of the chemical (including pH) changes that accompany spoilage as the 600 µg/ml level used in previous experiments.

Phase III Screens:

SEL fermentor experiments with decanoic acid were run at 37°C and 50 rpm using Park 500 SEL. Initial results indicate that decanoic acid exhibits the same antimicrobial effect in 5-liter fermentors as it does in 100 ml shake flask experiments. No change in pH, total reducing sugars, formic acid, acetic acid, NO₃⁻ and NH₃ were observed over a 24-48 hour period in the fermentor containing 600 µg/ml decanoic acid. Significant changes were observed for all the variables in the control fermentor containing the fresh, Park-500 SEL.

Miscellaneous:

A pilot plant run with decanoic acid has been completed (week of Oct. 10th). TC and 150B sheets +/- decanoic acid at final concentrations (on the sheet) of 1000, 2000 and 4000 ppm decanoic acid were produced for subjective evaluation.

C. Conclusions:

1. Potassium sorbate and benzoic acid were not as effective in the Phase I screen against PM-13 as were propylparaben or decanoic acid. Decanoic acid, at 50 µg/ml was found to be the most effective antimicrobial agent tested.
2. Three hundred to four hundred ppm decanoic acid in Park 500 SEL appears to be as effective in inhibiting spoilage in the Phase II screen as was the 600 ppm level decanoic acid previously reported.

D. Plans: October, 1988

1. Further evaluations of the fatty acid alcohols in conjunction with fatty acids will be conducted.
2. The C-10 and C-12 fatty acid alcohols will be tested in the Phase II, SEL shake-flask assay.
3. Evaluations of the C-10 and C-12 fatty acids and fatty acid alcohols will be initiated in the Phase III fermentor screen.
4. Dissolved oxygen, pH and temperature measurements will be made by Project 1730 and Park 500 personnel at selected locations in Line 2 of Park 500 in support of the design and establishment of a Park 500 model system at the R&D labs.